

# PATENT COOPERATION TREATY

PCT/EP94/03564

**PCT**

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark  
Office  
(Box PCT)  
Washington D.C. 20231  
United States of America

in its capacity as elected Office

Date of mailing:

29 May 1995 (29.05.95)

International application No.:

PCT/EP94/03564

Applicant's or agent's file reference:

K 2133 Wd

International filing date:

28 October 1994 (28.10.94)

Priority date:

28 October 1993 (28.10.93)

Applicant:

KERN, Andrea et al

1. The designated Office is hereby notified of its election made:



in the demand filed with the International Preliminary Examining Authority on:

24 April 1995 (24.04.95)



in a notice effecting later election filed with the International Bureau on:

2. The election ☒ was



was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO  
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1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Form PCT/IB/331 (July 1992)

Authorized officer:

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PATENT COOPERATION TREATY

PCT/EP94/03564

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NOTIFICATION CONCERNING  
DOCUMENT TRANSMITTED

From the INTERNATIONAL BUREAU

To:

United States Patent and Trademark  
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Washington D.C. 20231  
United States of America

in its capacity as elected Office

Date of mailing (day/month/year)

08 December 1995 (08.12.95)

International application No.

PCT/EP94/03564

International filing date (day/month/year)

28 October 1994 (28.10.94)

Applicant

DEUTSCHES KREBSFORSCHUNGSZENTRUM, STIFTUNG DES ÖFFENTLICHEN RECHTS et al

The International Bureau transmits herewith the following documents and number thereof:

\_\_\_\_\_ copy of the international preliminary examination report (Article 36(3)(a))

The International Bureau of WIPO  
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Form PCT/IB/310 (July 1992)

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000929840

## PATENT COOPERATION TREATY

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From the INTERNATIONAL BUREAU

NOTIFICATION OF THE RECORDING  
OF A CHANGE(PCT Rule 92bis.1 and  
Administrative Instructions, Section 422)

To:

DEUFEL, Paul  
Müller-Boré & Partner  
Grafinger Strasse 2  
D-81671 München  
ALLEMAGNEDate of mailing 20 September 1995 (20.09.95)  
(day/month/year)Applicant's or agent's file reference  
K 2133 WdInternational application No.  
PCT/EP94/03564

## IMPORTANT NOTIFICATION

International filing date  
(day/month/year) 28 October 1994 (29.10.94)

## 1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

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## 2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

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089/4905710 (gr. III)

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## 3. Further observations, if necessary:

## 4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned  
☐ the International Searching Authority ☒ the elected Offices concerned  
☒ the International Preliminary Examining Authority ☐ other:The International Bureau of WIPO  
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JOINT COOPERATION TREATY

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INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference <b>K 2133 Wd</b>	<b>FOR FURTHER ACTION</b> see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. <b>PCT/EP 94/03564</b>	International filing date (day/month/year) <b>28/10/94</b>	(Earliest) Priority Date (day/month/year) <b>28/10/93</b>
Applicant <b>DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG et al.</b>		

This international search report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This international search report consists of a total of 3 sheets.  
☒ It is also accompanied by a copy of each prior art document cited in this report.

1. ☐ Certain claims were found unsearchable (see Box I).
2. ☐ Unity of invention is lacking (see Box II).
3. ☒ The international application contains disclosure of a nucleotide and/or amino acid sequence listing and the international search was carried out on the basis of the sequence listing
  - ☐ filed with the international application.
  - ☒ furnished by the applicant separately from the international application,
    - ☐ but not accompanied by a statement to the effect that it did not include matter going beyond the disclosure in the international application as filed.
  - ☐ Transcribed by this Authority
4. With regard to the title,
  - ☒ the text is approved as submitted by the applicant.
  - ☐ the text has been established by this Authority to read as follows:
5. With regard to the abstract,
  - ☒ the text is approved as submitted by the applicant.
  - ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.
6. The figure of the drawings to be published with the abstract is:
  - Figure No.            ☐ as suggested by the applicant.
  - ☐ because the applicant failed to suggest a figure.
  - ☐ because this figure better characterizes the invention.
  - ☒ None of the figures.

## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 94/03564

## A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12Q1/70 G01N33/569 C07K14/015 C07K16/08

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 C12Q C07K G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OBSTETRICS AND GYNECOLOGY, vol.81, no.3, March 1993, NY US pages 402 - 408 ROGERS B B et al 'DETECTION OF HUMAN PARVOVIRUS B19 IN EARLY SPONTANEOUS ABORTUSES USING SEROLOGY HISTOLOGY ELECTRON MICROSCOPY IN-SITU HYBRIDIZATION AND THE POLYMERASE CHAIN REACTION' see the whole document	1,2,4, 7-12, 14-17
Y	---	3,5,13, 18-21
Y	WO,A,91 12269 (MIKROGEN MOLEKULARBIOLOGISCHE ENTWICKLUNGS-GMBH) 22 August 1991 see the whole document ---	3,5,13, 17-21
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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

8 February 1995

Date of mailing of the international search report

22-02-1995

Name and mailing address of the ISA

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GURDJIAN, D

# INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 94/03564

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 04330 (RIJKSUNIVERSITEIT TE LEIDEN) 4 April 1991	5, 13,
A	see abstract	17-21
	---	8
A	JOURNAL OF VIROLOGY, vol.45, no.2, 1983, WASHINGTON US pages 555 - 564 Srivastava, Arun et al 'Nucleotide sequence and organization of the adeno-associated virus 2 genome' -----	3

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/03564

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9112269	22-08-91	DE-A- 4003826	14-08-91
		AU-B- 650864	07-07-94
		AU-A- 7211591	03-09-91
		DE-D- 59101577	09-06-94
		EP-A- 0514413	25-11-92
		ES-T- 2052370	01-07-94
		JP-T- 5504143	01-07-93
WO-A-9104330	04-04-91	NL-A- 8902301	02-04-91
		EP-A- 0491824	01-07-92

**PCT**WORLD INTELLECTUAL PROPERTY ORGANIZATION  
International Bureau

## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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**93117452.8****28 October 1993 (28.10.93)****EP**(34) Countries for which the regional or  
international application was filed:**AT et al.**(71) Applicant (for all designated States except US): **DEUTSCHES  
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02 47, D-80059 München (DE).**(81) Designated States: **CA, JP, US, European patent (AT, BE, CH,  
DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).****Published***With international search report.**Before the expiration of the time limit for amending the  
claims and to be republished in the event of the receipt of  
amendments.*(54) Title: **ADENO-ASSOCIATED VIRUS - ITS DIAGNOSTIC USE WITH EARLY ABORTION**

## (57) Abstract

The present invention relates to a method of detecting a causative agent of the so-called spontaneous early abortion by investigating patients' samples for the presence of adeno-associated virus DNA (AAV DNA), or AAV antigen or antibodies, preferably of the IgM type, directed to AAV. Furthermore, the present invention relates to antibodies suitable for said method.



**Adeno-Associated Virus -  
Its Diagnostic Use with Early Abortion**

The present invention relates to a method of detecting a causative agent of the so-called spontaneous early abortion by investigating patients' samples for the presence of adeno-associated virus DNA (AAV DNA), or AAV antigen or antibodies, preferably of the IgM type, directed to AAV. Furthermore, the present invention relates to antibodies suitable for said method.

The adeno-associated viruses (AAV) which are human parvoviruses that depend on coinfecting helper viruses for their replication, are thought to be non-pathogenic (Siegl, G. et al. (1985), Intervirology, 23, pp. 61-73; Berns, K.I. et al. (1987), Adv. Virus Res., 32, pp. 243-306) but rather to exhibit tumorsuppressive properties (Rommelaere et al. (1991), J. Virol. Methods, 33, pp. 233-251). The virus may persist in infected individuals, possibly by integration of its DNA into specific chromosomal sites of the host cell genome as seen in cell culture. Recent studies of our laboratories have demonstrated that AAV is able to induce differentiation in a variety of cells of human and mouse origin (Klein-Bauernschmitt et al. (1992), J. Virol., 66, pp. 4191-4200) including embryonic stem cells. In the course of looking for putative targets of AAV infection, we analysed material from spontaneous abortion for the presence of AAV DNA using for example the polymerase chain reaction (PCR), the Southern blotting technique and the in situ hybridization technique. Additionally, we analysed serum samples from women with miscarriage and from other diseased or healthy women for the presence of antibodies to AAV using serological standard techniques such as enzyme linked immunosorbent assay (ELISA), fluorescenceimmuno assay (FIA), radioimmuno assay (RIA) or immunofluorescence assay (IFA).

Surprisingly, we found a significant correlation of both detectable AAV DNA in samples of abortion material and detectable IgM antibodies directed to AAV with the early abortion occurring during the first trimester of pregnancy.

Disclosure of the invention

Accordingly, the present invention relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

- a) hybridizing a probe for an AAV polynucleotide to nucleic acids of a sample of abortion material under conditions which allow the formation of a heteroduplex between an AAV nucleic acid and the probe, and
- b) detecting a polynucleotide duplex which contains the probe.

In a preferred embodiment of the present invention the method as mentioned above is a polymerase chain reaction (PCR), Southern blotting or in situ hybridization technique.

In another preferred embodiment of the present invention a hybridization technique is applied as described above, wherein one or more nucleic acid probes are used which are selected from the group consisting of the primers pan1, pan3, nest1 and nest2. In Figure 1 a schematic drawing of these primers, relative to the genome of the AAV type 2 (AAV-2) and the nucleotide sequences of the primers is presented.

The present invention further relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

- a) incubating a probe antibody directed to an AAV antigen with a sample of abortion material under conditions which allow the formation of an antigen-antibody complex, and
- b) detecting the antigen-antibody complex containing the probe antibody.

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In step (a) one or more probe antibodies can be used. These antibodies can be directed to e.g. an AAV capsid or a single protein thereof, particularly VP1, VP2 or VP3. Examples of these antibodies are the following monoclonals:

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A1; deposited at DSM under DSM ACC2195 on Oct. 13, 1994

A69; deposited at DSM under DSM ACC2196 on Oct. 13, 1994

B1; deposited at DSM under DSM ACC2197 on Oct. 13, 1994

A20; deposited at DSM under DSM ACC2194 on Oct. 13, 1994

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(see Table 1).

The antibodies as mentioned above are subject matter of the present invention.

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In a preferred embodiment of the present invention the method of antigen detection as mentioned above is an enzyme linked immunosorbent assay (ELISA), a radioimmuno assay (RIA), a fluorescence immuno assay (FIA) or an immunofluorescence assay (IFA).

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An example of the ELISA comprises the following steps:

- (a) providing a substrate carrying the monoclonal antibody A 20,
- (b) contacting the substrate of (a) with a sample of abortion material to get an antigen-antibody complex,
- 25 (c) contacting the complex of (b) with a polyclonal anti-AAV capsid antibody to get an antibody-antigen-antibody complex,
- (d) contacting the complex of (c) with an enzyme-labelled antibody directed to the polyclonal antibody of (c) to get a labelled complex of (c), and
- 30 (e) contacting the complex of (d) with an enzyme-label-indicator to indicate the presence of said complex.

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It is clear that the term "sample of abortion material" is only an example of materials which contain AAV capsids or parts thereof. Another examples are cells expressing recombinant AAV capsids or parts thereof.

5 The present invention, i.e. the antibodies alone or in combination with the AAV antigen detection method, is suitable to detect AAV capsids and/or parts thereof in any material.

10 Furthermore, the present invention relates to a method of detecting the causative agent of spontaneous abortion comprising the steps of

15 a) incubating a sample containing AAV or an antigenic part thereof with a sample suspected of containing anti-AAV antibodies under conditions which allow the formation of an antibody-antigen complex, preferably only containing antibodies of the IgM type, and

20 b) detecting an antibody-antigen complex, preferably IgM antibody-antigen complex, containing the probe antigen.

In step (a) the term "sample containing AAV or an antigenic part thereof" refers to AAV capsid proteins, particularly VP1, VP2 and/or VP3, preferably.

25 In another preferred embodiment of the present invention the method of detection of AAV specific antibodies, particularly IgM antibodies, is an ELISA, a RIA, a FIA or an IFA.

An example of the ELISA comprises the following steps:

- 30 (a) providing a substrate carrying an anti-human IgM antibody,  
(b) contacting the substrate of (a) with a patient's bodyfluid to get an antibody-antibody complex,

- 5 -

- (c) contacting the complex of (b) with recombinant VP1, VP2 and/or VP3 to get a VP-antibody-antibody complex,
- (d) contacting the complex of (c) with an anti-VP-antibody to get an anti-VP-antibody-VP-antibody-antibody complex,
- (e) contacting the complex of (d) with an enzyme-labelled antibody directed to the anti-VP-antibody of (d) to get a labelled complex of (d), and
- (f) contacting the complex of (e) with an enzyme-label-indicator to indicate the presence of said complex.

It is evident that persisting anti-AAV IgM/IgG titers in serum are associated with predisposition to early abortions. Thus, the present invention can also be used for effective risk factor screening, development of methods for prevention of pregnancy failure, and information of patients about the risks of pregnancy failure.

Furthermore, the present invention relates to a kit for detecting the causative agent of spontaneous abortion by hybridization as described above, comprising a probe for an AAV polynucleotide in a suitable container.

The present invention further relates to a kit for detecting the causative agent of spontaneous abortion by immunological antigen detection as described above, comprising a probe antibody directed against an AAV antigen in a suitable container.

The present invention further relates to a kit for detecting the causative agent of spontaneous abortion by immunological antibody detection as described above, comprising AAV or an antigenic part thereof in a suitable container.

#### Modes for carrying out the invention:

The art is rich in methods available to the man of the art in recombinant

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nucleic acid technology, microbiology and immunobiology for carrying out the present invention. Detailed descriptions of all of these techniques will be found in the relevant literature. See for example Maniatis, Fritsch & Sambrook: Molecular Cloning: A Laboratory Manual (1989); DNA Cloning, Vol. I and II (D.N. Glover ed., 1985); Oligonucleotide Synthesis (M.J. Gait ed., 1984); Nucleic Acid Hybridization (B.D. Hames & S.J. Higgins eds., 1984); Animal Cell Culture (R.I. Freshney ed., 1986); J.D. Watson, M. Gilman, J. Witkowski, M. Zoller: Recombinant DNA, Second Edition (1992); Immunochemical Methods in Cell and Molecular Biology (Academic Press, London, 1987); Protein Purification: Principles and Practice, Second Edition (Springer Verlag, N.Y.); Handbook of Experimental Immunology, Vol. I-IV (D.M. Weir and C.C. Blackwell eds., 1986); Immunoassay: A Practical Guide (D.W. Chan and M.T. Perlstein eds., 1987); ELISA and Other Solid Phase Immunoassays: Theoretical and Practical Aspects (D.M. Kemeny and S.J. Challacombe eds., 1988); Principles and Practice of Immunoassay (C.P. Price and D.J. Newman eds., 1991).

More detailed information on specific methodological aspects of AAV, such as cell culture, virus growth, virus purification, isolation of proteins, can be found in the relevant literature, e.g. Handbook of Parvoviruses, Vol. I and II CRC Press, Boca Raton, Florida, Ed. P. Tijssen; Ruffing, M. et al. (1992), J. Virol., 66, pp. 6922-6930.

All reagents such as antigens, antibodies, probe antigens, probe antibodies, nucleic acid probes, primers and auxiliary reagents necessary to perform an immunoassay or a hybridization assay, possibly using amplification techniques for improved sensitivity may be filled into suitable containers or coated to any solid phase such as plastic, glass and cells, and packaged into kits together with instructions for conducting the test.

The present invention is exemplified by the following examples.

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Example 1:

Detection by Polymerase-Chain-Reaction (PCR) - analysis of AAV DNA in biological, e.g., curettage material of spontaneous abortion.

The primers used in PCR (pan1, pan3) and nested PCR (nest1, nest2), respectively, were designed to hybridize to sequences of AAV-2 and AAV-5 DNA by allowing mismatches not leading to amplification of other (e.g. cellular) DNA sequences. The amplified products are distinguishable by Southern blot experiments. The primers were prepared according to standard procedures.

The primers were designed displaying mismatches (underlined) as shown below:

----- AACTGGACCAATGAAAACTTTCC ----- pan1

1386 TCGGTAAACTGGACCAATGAGAACTTTCCCTTCAAC AAV-2

130 TCGGTAAACTGGACCAATGAAACTTTCCCTTCAAC AAV-5

---

AAAAAGTCTTTGACTTCCTGCTT pan3

1729 AAAAAGTCTTTGACTTCCTGCTT AAV-2

472 AAAAAGTCCTTGACTTCCTGCTT AAV-5

DNA prepared from histological sections (5  $\mu$ m, of fresh or fixed, paraffin-embedded, deparaffinated material [Methods as described by D.H. Wright and M.M. Manos in "PCR Protocols, A Guide to Methods and Applications", edited by M.A. Innis, D.H. Gelfand, J.J. Snoisky and T.L. White, Chapter 19, pp. 153-158; Academic Press, New York, 1990] were analysed by PCR using

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the primers pan1 and pan3 combined, followed in AAV positive cases by a repetition of the PCR (to confirm specificity) using the (internal) primers nest1 and nest2 (see Figure), respectively. PCRs were performed for 40 cycles (one cycle = 92 °C, 1 min; 62 °C, 4 min; 92 °C, 15 sec) (van den Brule et al., (1989) J. Med. Virol., 29, pp. 20-27). Amplified products were characterized by electrophoretic separation (2 % agarose gel) and blotting onto a nylon membrane (Gene Screen, NEN, Dupont, Dreieich, Germany) followed by hybridization at high stringency with <sup>32</sup>P-labelled probes (labelled using the Megaprime™ DNA Labelling System, Amersham, UK) of AAV-2 (pTAV2 [Heilbronn et al. (1990), J. Virol., 64, pp. 3012-3018]) or of AAV-5. This probe was cloned from DNA from purified AAV-5 virions, propagated with adenovirus type 12 and purified as described in de La Maza and Carter (1980), J. Virol., 33, pp. 1129-1137 and in Rose (1974) Parvovirus Reproduction, pp. 1-61; In: H. Fraenkel-Conrat and R.R. Wagner, eds., Comprehensive Virology, Plenum Press, New York.

#### Example 2:

##### Detection by Southern Blotting analysis of AAV DNA in fresh curettage material

Genomic DNA was isolated using standard procedures with minor modification (Laird et al. (1991), Nucl. Acids Res., Vol. 19, pp. 4293-4294) and digested with restriction enzymes allowing analysis of characteristic restriction sites within the AAV genome. After separation through 0,8 % agarose gels, DNA fragments were blotted onto Nylon membranes (Gene Screen) and hybridized AAV-2 DNA (pTAV2, see Example 1) or specific AAV-5 DNA (see Table 2) labelled by random priming with [ $\alpha$ -<sup>32</sup>P] dCTP (Amersham, Braunschweig, Germany).



Example 3:

Detection of AAV DNA by in situ hybridization in sections of biopsy material, e.g. curettage from spontaneous abortion

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In situ hybridization was performed as described (Tobiasch et al. (1992) Differentiation, 50, pp. 163-178), however, with the modification that AAV-2 DNA was detected by RNA-DNA hybridization. After DNase treatment, the probes were subjected to limited alkaline hydrolysis. Upon linearisation of the plasmid pTAV2 (Heilbronn et al. (1990), see above) with EcoRV, riboprobes were obtained and labelled with [<sup>35</sup>S]-UTP by in vitro transcription with T7 RNA polymerase (method as described in Boehringer Mannheim Procedure supplied with the "SP6/17 Transcription Kit"). Prior to hybridization, both probe and target DNA were denatured (93 °C, 10 min). For in situ hybridization with [<sup>32</sup>P]-UTP labelled probes, the protocol was as described in Dürst et al. (1992) Virology, 189, pp. 132-140.

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Example 4

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Provision of antibodies directed to AAV capsid proteins

In order to generate monoclonal antibodies directed to AAV capsid proteins two BALB/c mice were injected subcutaneously (s.c.) with 150 µl of a mixture of gel purified recombinant capsid proteins in PBS containing 100 µg each of VP1, VP2 and VP3, mixed with an equal volume of complete Freund's adjuvant. After four weeks the mice were boosted s.c. with 25 µg of purified UV-inactivated AAV-2 in 50 µl PBS and 50 µl incomplete Freund's adjuvant. After four weeks the mice were injected intraperitoneally (i.p.) each with 10 µg of UV-inactivated AAV-2 in 100 µl PBS. Three days later one mouse was killed and the spleen cells were fused with X63/Ag8 cells according to standard procedures (Harlow, E. and Lane, D. (1988), Cold

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- 10 -

Spring Harbor Laboratory, Antibodies, A laboratory manual). Resultant hybridoma culture supernatants were screened by Western blotting, immunofluorescence and ELISA. The second mouse was immunized six months later with 100  $\mu$ g of purified VP3 in PBS (i.p.) and monoclonal antibodies were prepared as described above.

#### Example 5:

#### ELISA for the detection of IgG antibodies directed to AAV

96-well microtiterplates (Nunc, Denmark) were coated with 50  $\mu$ l CsCl-gradient purified AAV 2 (dilution 1:1000 in 0,05 M carbonate-buffer pH 9,6) or with 50  $\mu$ l recombinant AAV 2 capsid proteins VP1-3 (1:8000 in 0,05 M carbonate-buffer) and incubated over night at RT. Plates were washed twice (washing buffer: PBS, 0,05 % Tween 20) and human sera were added (50  $\mu$ l/well, dilutions 1:25 to 1:800, dilution buffer: PBS, 2 % BSA, 0,05 % Tween 20) and incubated for 1 h at 37 °C in a wet chamber. After washing plates were incubated with 50  $\mu$ l/well peroxylase conjugated monkey anti-human IgG antibody (1:2000) for 45 minutes at 37 °C in a wet chamber. Plates were washed four times and 50  $\mu$ l substrate solution ( 5 mg OPD in 25 ml 0,1 M citratebuffer pH 5,0 + 10  $\mu$ l H<sub>2</sub>O<sub>2</sub> 35 %) was added. Plates were stored for 10-15 minutes in the dark and the reaction was stopped with 50  $\mu$ l 1N H<sub>2</sub>SO<sub>4</sub>/well. Extinctions were measured at 492 nm in a Titertek photometer. Background signal was determined by measuring the extinction without adding human sera and was subtracted on every well (background signal extinction ranged from 0,035 to 0,05).

#### Example 6:

#### ELISA for the detection of IgM antibodies directed to AAV

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**Version A**

Plates were coated as described in Example 4. Human sera were added after they had been treated according to the following absorption protocol in order to eliminate remaining IgG-antibodies : 20  $\mu$ l absorption reagent (FREKA-Fluor, Fresenius, Germany) were diluted with 25  $\mu$ l PBS and 5  $\mu$ l of human serum was added. Absorption was performed for at least 15 minutes at RT, and subsequently sera were tested at dilutions from 1:100 to 1:800. Incubation was performed for 1 h at 37 °C in a wet chamber and after washing 50  $\mu$ l/well peroxidase conjugated goat anti human IgM antibody (1:2000 in PBS/2 % BSA/0,05 % TWEEN 20) were added. Plates were incubated for 45 minutes at 37 °C and washed four times. The OPD reaction and photometric evaluation were performed as described in Example 5.

**Version B** **$\mu$ -capture ELISA****Plate Coating**

Rabbit anti-human IgM antibody (DAKO) was first denatured at a protein concentration of 600 $\mu$ g/ml, incubating for 30 min at RT in 50mM glycine/HCl pH 2,5 containing 100 mM NaCl then neutralized with 1 M Tris base. The denatured antibody was then desalted by passing the solution over a Sephadex PD 10 column equilibrated in the coating solution (10mM Tris/HCl pH 8,5 containing 100 mM NaCl). The sample was eluted from the column in the same buffer. The solution was adjusted to a protein concentration of 6 $\mu$ g/ml by dilution in coating buffer and 200 $\mu$ l added to each well on a polystyrene microtiter plate (NUNC immuno flat-bottomed well). The plate was incubated at 37°C for 24 h in a humid atmosphere, contents decanted and wells washed 4 times with 250 $\mu$ l/well of Tris-buffered saline (TBS) (0,02 M Tris/HCl pH 7,4, 0,15 M NaCl) containing 0,05 % Tween 20 (wash buffer). The wells were then blocked with TBS containing 1 % Tween 20 and 5 % Sucrose (blocking solution) by incubating at 4°C followed by 2 washings in wash buffer (TBS containing 0,05% Tween 20).

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**Assay**

The second step in the ELISA involved contacting patients' sera with the antibody-coated plate. During incubation, IgM was immunologically bound to the solid-phase antibody. After removal of the unbound material and washing of the microtiter plates, the plates were incubated with purified recombinant AAV nucleocapsid proteins VP1, VP2 and VP3. After removal of the unbound material and washing of the microtiter plates, complexes of human IgM antibody-VP complexes were detected by incubation with the A1, A69 and B1 antibodies. Unbound monoclonal antibodies were removed by aspiration and the plates were washed. The bound monoclonal antibodies were detected by incubating the plates with goat anti-mouse immunoglobulin antibodies conjugated to horseradish peroxidase (HRP). Following removal of unbound conjugate by washing, a solution containing  $H_2O_2$  3.3', 5.5' tetramethylbenzidine (TMB) was added. Reactions were stopped after a suitable interval by addition of sulfuric acid. The cutoff value of the ELISA was calculated as the average optical density of five negative samples plus 3 standard deviations (to correct for any aspecific binding). Samples giving absorbance values higher than the cutoff were considered positive.

Specifically, the anti-human IgM on the plate was reacted with serum by adding 100 $\mu$ l of serum samples diluted 1:200 in TBS containing 10 mg/ml bovine serum albumin, and incubating the serum-containing wells for 1 h at room temperature. After incubation, the serum samples were removed by aspiration and the wells were washed 5 times with washing solution (TBS + 0,05% Tween 20). Aliquots of 100 $\mu$ l of the VP1, VP2 and VP3 antigen mixture (conc of 10-10 nM VP1, VP2 and VP3) were added to each well and the plates were incubated at room temperature at least 2 h, followed by removal of excess probe by aspiration and 5 washes with TBS + 0,05 % Tween 20. Bound VP1, VP2 and VP3 was detected by addition of 100 $\mu$ l of a mixture of hybridoma supernatants from A1, A69 and B1 monoclonal antibodies producing hybridomas (antibody conc 1-10 nM), followed by 5 standard washes of the plates with TBS + 0,05% Tween 20. Monoclonal

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antibody binding was detected by addition of 200  $\mu$ l of 1 1/2000 dilution of sheep anti-mouse IgG horseradish peroxidase-conjugated antibody (Dako, Hamburg/Germany) and incubated for 1,5 h at room temperature, followed by 5 standard washings of the plate. Enzyme activity was revealed by addition of 100  $\mu$ l of a solution of TMB (Serex, Maywood, N.J./USA). The plate was incubated until the desired color development was reached and terminated by addition of 50  $\mu$ l 2N sulfuric acid. Optical densities ( $OD_{450}$ ) of negative and positive control sera as well as samples were determined. The cutoff value as calculated from five negative sera was  $OD_{450} = 0,40$ .

#### Example 7:

#### ELISA for the detection of AAV capsids

##### Plate Coating

100  $\mu$ l of the A20 antibody (see above) equilibrated in coating buffer solution (50 mM  $NaHCO_3$  pH 9.6 and adjusted to a protein concentration of 1,5 ng/ml) was added to each well on a polystyrene microtiter plate (NUNC immuno flat-bottomed well). The plate was incubated at 4 °C for 24 h, contents decanted and wells, washed 5 times with 250  $\mu$ l/well of phosphate-buffered saline (PBS) (wash buffer). The wells were blocked with 260  $\mu$ l of 3 % BSA in PBS (blocking solution) by incubating at least 30 minutes at room temperature followed by 6 washings in wash buffer.

##### Assay

A standard curve within the range of 10 - 10 000 capsids/ml was prepared by diluting AAV capsids in standard dilution solution containing PBS.

Unknown samples were diluted as appropriate in diluent solution and 100  $\mu$ l added to the test wells. When tissue culture supernatants were to be assayed, 100  $\mu$ l of a 1:10 to 1:10<sup>8</sup> dilution was to be added to the test well.

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5 The plate was incubated for 3 h at room temperature. The plate was washed  
5 times in wash buffer and 100  $\mu$ l rabbit anti-AAV-polyclonal antiserum at a  
dilution of 1/1000 in 3 % BSA in PBS added to each well. The plate was  
10 incubated at room temperature for 2 h as previously and then washed 5  
times in PBS Tween. AAV capsid was detected by addition of 100  $\mu$ l of a  
1/2000 dilution of a goat anti-rabbit IgG myeloperoxidase-conjugated anti-  
body prepared in antibody diluent and incubated for 1 h at room temperature  
followed by 5 standards washes of the plate. Enzyme activity was revealed  
by addition of 100  $\mu$ l of a 0,1 mg/ml solution of tetramethylbenzidine (TMB)  
15 prepared in 0,1 M Na-acetate buffer pH 6 to each well. The plate was incu-  
bated at room temperature until the desired color development was reached,  
longer incubation periods being necessary to detect lower concentration  
ranges, i.e. standards less than 10 capsids/ml. The concentration of  
unknown samples was determined by comparison of their optical density to  
the standard curve.

#### Example 8:

##### Detection of AAV-DNA in curettage materil of spontaneous absorption

20 A total of 50 samples of curettage material of spontaneous absorption were  
analysed for the presence of AAV DNA either by PCR or Southern Blotting or  
both. 41 samples were from abortions in the first and 9 samples from abor-  
tions in the second and third trimester of pregnancy.

25 Among the 41 samples taken during the first trimester of pregnancy, 14 con-  
sisted of fresh material that could be tested by Southern Blotting, by which  
method 9 samples were shown to be positive. All other samples tested were  
sections from paraffin-embedded tissues, that were analysed by PCR. Among  
30 these, 30 samples were from abortions in the first trimester of pregnancy, of  
which 12 samples were shown to be positive for AAV DNA. All of the 9  
samples from the second or third trimester of pregnancy were negative by

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PCR.

5 Thus, in 21 of 41 samples, i.e. 50 % of spontaneous abortions in the first trimester of pregnancy AAV specific DNA sequences could be detected, whereas 9 spontaneous abortions in the second or third trimester were negative (see Table 3).

Example 9:

10 A total serum of 148 serum samples drawn from healthy probands, diseased patients with various syndromes being unrelated to abortion, and pregnant women with spontaneous abortion during the first trimester of pregnancy were tested for antibodies directed to AAV.

15 The results obtained are displayed in Table 4. Generally, the prevalence of specific IgG antibodies was quite high, between 62 and 100 % in the different groups of probands/patients. However, specific IgM antibodies were shown to be significantly correlated with "pregnancy problems".

Table 1

Term	Subtype	Epitope	Western Blotting	Immuno- Precipitation	Immuno- Fluorescence	Characteristics
A1	IgG2a	between aa 1 - 104	+ specific recognition of VP1	+	+	recognition of monomeric and oligomeric VP1
A69	IgG1	between aa 105 - 136	+ specific recognition of VP1 and VP2	++	++	recognition of monomeric and oligomeric VP1 and VP2
B1	IgG1	between aa 136 - 669	++ recognition of VP1, VP2 and VP3	++	++	recognition of monomeric and oligomeric VP1, VP2 and VP3
A20	IgG3	presumable conformation	- (negativ)	+++	++	preferable recog- nition of AAV capsid, no reaction with recombinant monomeric capsid protein

aa: amino acid6



1 Table 2

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5 388 bp part of BamH1b fragment of AAV5

10 487 TCAATCAGGTGCCGGTGACTCACGAGTTTAAAGTTCCCAGGGAATTGGCGGGAACATAAG  
AGTTAGTCCACGGCCACTGAGTGCTCAAATTTCAAGGGTCCCTTAACCGCCCTTGATTTC 546

547 GGGCGGAGAAATCTCTAAAACGCCCCTGGGTGACGTCACCAATACTAGCTATAAAAGTC  
CCCGCCTCTTTAGAGATTTTGCGGGTGACCCACTGCAGTGGTTATGATCGATATTTTCAG 606

15 607 TGGAGAAGCGGGCCAGGCTCTCATTGTTCCTGAGACGCCTCGCAGTTCAGACGTGACTG  
ACCTCTTCGCCCCGGTCCGAGAGTAAACAAGGGCTCTGCGGAGCGTCAAGTCTGCACTGAC 666

20 667 TTGATCCCGCTCCTCTGCGACCGCTCAATTGGAATTCAAGGTATGATTGCAAATGTGACT  
AACTAGGGCGAGGAGACGCTGGCGAGTTAACCTTAAGTTCCATACTAACGTTTACACTGA 726

25 727 ATCATGCTCAATTTGACAACATTTCTAACAAATGTGATGAATGTGAATATTTGAATCGGG  
TAGTACGAGTTAAACTGTTGTAAAGATTGTTTACACTACTTACACTTATAAACTTAGCCC 786

787 GCAAAAATGGATGTATCTGTCACAATGTAACCTCACTGTCAAATTTGTCATGGGATTCCCC  
CGTTTTTACCTACATAGACAGTGTTACATTGAGTGACAGTTTAAACAGTACCCTAAGGGG 846

30 847 CCTGGGAAAAGGAAAACCTTGTCAGATTT  
GGACCCTTTTCCTTTTGAACAGTCTAAA 874

35

Table 3

5

Prevalence of AAV DNA in curettage materials

10

15

20

Diagnosis / Pathology	Detection of AAV DNA by (n AAV positive / number analy		
	PCR	Southern Blotting	
spontaneous abortion (1st trimester of pregnancy)	12/30	9/14	2
abortion 2nd trimester	0/3	n.d.	
abortion 3rd trimester or placenta post partum	0/6	n.d.	

n.d. = not done;

\* = 3 samples positive with PCR were tested by Southern  
analysis

## CLAIMS:

1. A method of detecting the causative agent of spontaneous abortion comprising the steps of
  - (a) hybridizing a probe for an AAV polynucleotide to nucleic acids of a sample of abortion material under conditions which allow the formation of a heteroduplex between an AAV nucleic acid and the probe, and
  - (b) detecting a polynucleotide duplex which contains the probe.
2. The method according to claim 1, which is a PCR, Southern blotting or an in situ hybridization technique.
3. The method according to claim 1, wherein one or more probes are used which are selected from the group consisting of the primers pan1, pan3, nest1 and nest2.
4. A method of detecting the causative agent of spontaneous abortion comprising the steps of
  - (a) incubating a probe antibody directed to an AAV antigen with a sample of abortion material under conditions which allow the formation of an antigen-antibody complex, and
  - (b) detecting the antigen-antibody complex containing the probe antibody.
5. The method according to claim 4, wherein the probe antibody is A1 (DSM

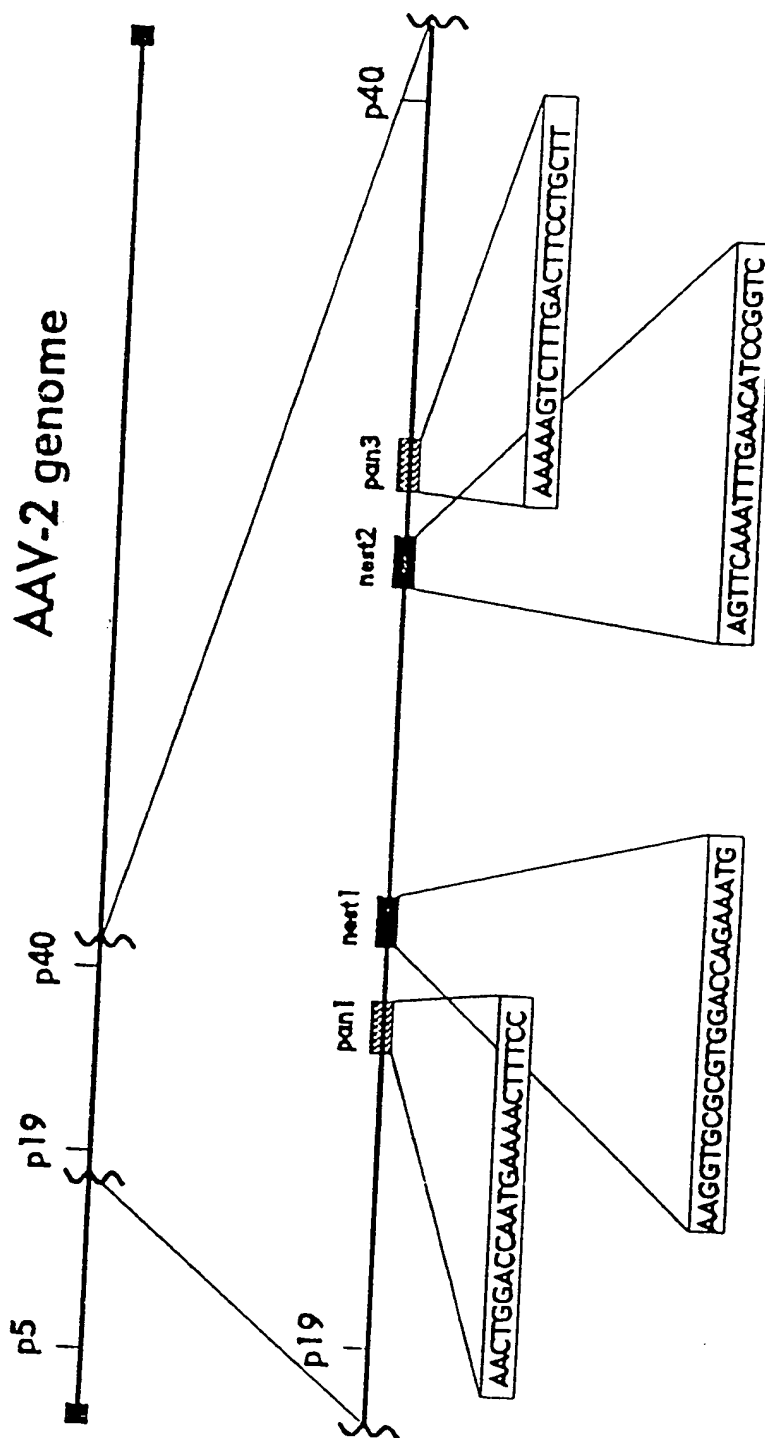
- 21 -

ACC2195, deposited on 13. 10. 1994), A20 (DSM ACC2194, deposited on 13. 10. 1994), A69 (DSM ACC2196, deposited on 13. 10. 1994) and/or B1 (DSM ACC2197, deposited on 13. 10. 1994).

6. The method according to claim 4 or 5, which is an ELISA, a RIA, a FIA or an IFA.
7. A method of detecting the causative agent of spontaneous abortion comprising the steps of
  - (a) incubating a sample containing AAV or an antigenic part thereof with a sample suspected of containing anti-AAV antibodies under conditions which allow the formation of an antibody-antigen complex, and
  - (b) detecting the antibody-antigen complex, containing the probe antigen.
8. The method according to claim 7, wherein the antigenic part of AAV is VP1, VP2 or VP3.
9. The method according to claim 7 or 8, wherein the antibody in the antibody-antigen complex is of the IgM type.
10. The method according to one of claim 7 to 9, which is an ELISA, a RIA, a FIA or an IFA.
11. A kit for performing the method according to claim 1, comprising a probe for an AAV polynucleotide in a suitable container.
12. A kit for performing the method according to claim 4, comprising a probe antibody directed to an AAV antigen in a suitable container.

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13. The kit according to claim 12, wherein the probe antibody is A1 (DSM ACC2195, deposited on 13.10.1994), A20 (DSM ACC2194, deposited on 13.10.1994), A69 (DSM ACC2196, deposited on 13.10.1994 and/or B1 (DSM ACC2197, deposited on 13.10.1994).
14. A kit for performing the method according to claim 7, comprising AAV or an antigenic part thereof in a suitable container.
15. The kit according to claim 14, wherein the antigenic part of AAV is VP1, VP2 and/or VP3.
16. Antibody directed to an AAV antigen.
17. Antibody according to claim 16, wherein the antibody is directed to an AAV capsid or a protein thereof.
18. Antibody according to claim 17, wherein the antibody is A1 (DSM ACC2195, deposited on 13.10.1994).
19. Antibody according to claim 17, wherein the antibody is A20 (DSM ACC 2194, deposited on 13.10.1994).
20. Antibody according to claim 17, wherein the antibody is A69 (DSM ACC2196, deposited on 13.10.1994).
21. Antibody according to claim 17, wherein the antibody is B1 (DSM ACC2197, deposited on 13.10.1994).

Figur 1

# INTERNATIONAL SEARCH REPORT

International Application No  
**PCT/EP 94/03564**

**A. CLASSIFICATION OF SUBJECT MATTER**  
**IPC 6 C12Q1/70 G01N33/569 C07K14/015 C07K16/08**

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)  
**IPC 6 C12Q C07K G01N**

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	OBSTETRICS AND GYNECOLOGY, vol.81, no.3, March 1993, NY US pages 402 - 408	1,2,4, 7-12, 14-17
Y	ROGERS B B et al 'DETECTION OF HUMAN PARVOVIRUS B19 IN EARLY SPONTANEOUS ABORTUSES USING SEROLOGY HISTOLOGY ELECTRON MICROSCOPY IN-SITU HYBRIDIZATION AND THE POLYMERASE CHAIN REACTION' see the whole document	3,5,13, 18-21
Y	WO,A,91 12269 (MIKROGEN MOLEKULARBIOLOGISCHE ENTWICKLUNGS-GMBH) 22 August 1991 see the whole document	3,5,13, 17-21
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☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

\* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

**8 February 1995**

Date of mailing of the international search report

**22 -02- 1995**

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## INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 94/03564

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO,A,91 04330 (RIJKSUNIVERSITEIT TE LEIDEN) 4 April 1991	5,13,
A	see abstract	17-21
	---	8
A	JOURNAL OF VIROLOGY, vol.45, no.2, 1983, WASHINGTON US pages 555 - 564 Srivastava, Arun et al 'Nucleotide sequence and organization of the adeno-associated virus 2 genome' -----	3



# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 94/03564

PC1/EP 94/03564

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A-9112269	22-08-91	DE-A- 4003826	14-08-91
		AU-B- 650864	07-07-94
		AU-A- 7211591	03-09-91
		DE-D- 59101577	09-06-94
		EP-A- 0514413	25-11-92
		ES-T- 2052370	01-07-94
		JP-T- 5504143	01-07-93
-----			
WO-A-9104330	04-04-91	NL-A- 8902301	02-04-91
		EP-A- 0491824	01-07-92
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# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

REC'D 06 DEC. 1995

Applicant's or agent's file reference <b>K 2133 Wd</b>	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. <b>PCT/EP 94/ 03564</b>	International filing date (day/month/year) <b>28/10/1994</b>	Priority date (day/month/year) <b>28/10/1993</b>
International Patent Classification (IPC) or national classification and IPC <b>C12Q1/70</b>		
Applicant <b>DEUTSCHES KREBSFORSCHUNGSZENTRUM STIFTUNG et al.</b>		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


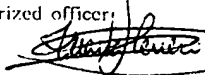
2. This REPORT consists of a total of 5 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consists of a total of \_\_\_\_\_ sheets.

3. This report contains indications and corresponding pages relating to the following items:

- I ☒ Basis of the report
- II ☐ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☒ Certain defects in the international application
- VIII ☐ Certain observations on the international application

Date of submission of the demand <b>24/04/1995</b>	Date of completion of this report <b>04. 12. 95</b>
Name and mailing address of the IPEA/  European Patent Office D-80298 Munich Tel. (+49-89) 2399-0, Tx: 523656 cpmu d Fax: (+49-89) 2399-4465	Authorized officer:  <b>M. Herrero</b>  Telephone No.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.  
PCT/EP94/03564

I. Basis of the report

1. This report has been drawn up on the basis of (Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.):

☒ the international application as originally filed.

☐ the description, pages \_\_\_\_\_, as originally filed,  
pages \_\_\_\_\_, filed with the demand,  
pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_,  
pages \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

☐ the claims, Nos. \_\_\_\_\_, as originally filed,  
Nos. \_\_\_\_\_, as amended under Article 19,  
Nos. \_\_\_\_\_, filed with the demand,  
Nos. \_\_\_\_\_, filed with the letter of \_\_\_\_\_,  
Nos. \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

☐ the drawings, sheets/fig \_\_\_\_\_, as originally filed,  
sheets/fig \_\_\_\_\_, filed with the demand,  
sheets/fig \_\_\_\_\_, filed with the letter of \_\_\_\_\_,  
sheets/fig \_\_\_\_\_, filed with the letter of \_\_\_\_\_.

2. The amendments have resulted in the cancellation of:

☐ the description, pages \_\_\_\_\_.  
☐ the claims, Nos. \_\_\_\_\_.  
☐ the drawings, sheets/fig \_\_\_\_\_.

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.  
PCT/EP94/03564

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step and industrial applicability; citations and explanations supporting such statement

1. STATEMENT

Novelty (N)	Claims 1-21	YES
	Claims	NO
Inventive Step (IS)	Claims 1-15, 18-21	YES
	Claims 16-17	NO
Industrial Applicability (IA)	Claims 1-21	YES
	Claims	NO

2. CITATIONS AND EXPLANATIONS

2.1 After comparison with the documents cited in the International Search Report the subject-matter according to present Claims 1-15 and 18-21 is considered to meet the novelty and inventive steps criteria set forth in Article 33(2) and (3) PCT, insofar as the finding on which the present invention is based, namely the significant correlation of both detectable AAV DNA in samples of abortion material and detectable IgM antibodies directed to AAV with the early abortion occurring during the first trimester of pregnancy as hereby disclosed (see page 2, last paragraph), appears to be neither anticipated nor suggested by the available prior art.

2.2 Notwithstanding the foregoing, the application does not satisfy the criterion set forth in Article 33(3) PCT because the subject-matter of Claims 16 and 17 does not involve an inventive step (Rule 65(1)(2) PCT).

Although no particular antibody directed against an AAV antigen is disclosed in the prior art cited in the

International Search Report, and thus the novelty criterion set forth in Art. 33(2) PCT would appear to be met by Claims 16 and 17, it cannot be ruled out the fact that such antibodies may have been disclosed in documents of the related field (see e.g. paragraph 2, on page 2 of the present description) which are not available to this Examining Authority. In any case, no inventive contribution as required by Art. 33(3) PCT can be regarded in claiming per se generic antibodies which are exclusively defined by the characterizing feature of being directed against known available antigens, for example in this case, an AAV VP1, VP2 or VP3 nucleocapsid antigen.

- 2.3 The subject-matter of Claims 1-21 seemingly finds a direct industrial applicability, e.g., in the clinical diagnostic field (Article 33(4) PCT).

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

Intern. application No.  
PCT/EP94/03564

VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:

1. The terms "Tween" (page 11, lines 16 and 18; page 12, lines 10, 28, 29 and 31; page 13, lines 25, 29 and 32), "Titertek" (page 11, line 25) and "Sephadex" (page 12, lines 20-21) appear to be registered trade marks but have not been identified as such.
2. The following expressions appear to contain typing mistakes:
  - Page 7, line 4 that reads ... A Loboratory Manual...
  - Page 11, line 1 that reads ... A laboratory mannual...
  - Page 15, line 19 that reads, ...in curettage materil...
  - Page 17, line 10 that reads, aa: amino acid<sub>6</sub>